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# THE ACTION OF TYPHOID BACILLI ON MILK AND ITS PROBABLE RELATION TO A SECOND CARBOHYDRATE IN THAT FLUID.

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It seems to be generally accepted, and stated in the most recent text-books, that the typhoid bacillus is peculiar as a producer of acid, more especially in milk and in the litmus whey of Petruschky,[1] made from it by removing casein and fat. Apparently in conflict with this view is the statement I have repeatedly made that lactose is not acted upon by typhoid bacilli when dissolved in dextrose-free bouillon. Drawn into some experiments touching this subject recently for another purpose, I studied series of cultures in fermentation tubes to explain, if possible, this discrepancy. The figures I shall give show that while typhoid bacilli produce acid in milk, they produce neither more nor less than other related bacilli which do not attack lactose. They do, however, differ from them in one important particular, — that of alkali-production, — which is responsible for these seemingly conflicting views. There is also another interesting question involved which concerns the nature of the substance in milk yielding the acid. This I take to be dextrose (glucose) or some carbohydrate closely resembling it. In order that the method of proof used may clearly be understood, I will briefly quote the principles upon which it is based.

1. Many bacteria, while acting on any carbohydrates present in culture fluids to form acids, at the same time produce in the presence of air an alkali. The alkali production is

[1] Bakterio-chemische Untersuchungen. Centralbl. f. Bakteriologie, VII. (1890), pp. 1 and 49.

linked to the multiplication of bacteria, and seems to vary directly with the vigor of growth.

2. This double process, which, in the presence of air, may be masked by mutual neutralization of the acid and alkaline bodies formed, is best studied in the fermentation tube, because in the closed branch alkali production is inhibited and acid production unchecked.

3. Fluids free from carbohydrates do not support life in the closed branch, *i.e.*, under anaërobic conditions.

4. Peptones do not yield acids. This seems restricted to those sugars which the bacteria under observation are able to decompose.

5. When a fermentescible sugar is present, the production of acid goes on until the sugar is used up, or, if the latter is present in excess, until growth is inhibited by the acid formed.

6. The amount of sugar which can be decomposed by bacteria before inhibition begins varies somewhat with the species, but probably does not exceed 0.5 per cent. unless neutralizing substances like $CaCO_3$ be added. These propositions I have repeatedly demonstrated by series of experiments which have been published in detail.[1]

At the outset it is of importance to examine briefly what action typhoid bacilli have upon sugars. That they act vigorously upon dextrose is known, but because gases are not set free the acid production is frequently overlooked.

March 7, 1898. In the closed branch of two fermentation tubes containing a one-per-cent. dextrose bouillon, and inoculated eight days ago with typhoid II. and III., the acid reaction of the bouillon had risen from 1.5 to 5.38 and 5.43 respectively.[2]

March 30. A five-day culture of typhoid bacilli in fluid of the same composition gave the following figures: Open bulb, —4.86; branch, —4.43.[3]

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[1] See Centralblatt f. Bakteriologie, VIII., p. 389; XI., p. 367; XIV., p. 864; XVIII., p. 1; XXII., p. 45.

[2] These figures signify that so much of a normal solution of acid or alkali percentum is required to bring the culture fluid to the phenolphthallein neutral point. For the method of titration employed, see "Procedures Recommended for the Study of Bacteria," Concord, N.H., 1898, p. 18.

[3] — signifies here acidity; + alkalinity.

A simple demonstration of the selective action of typhoid bacilli on dextrose may be made by adding 0.5 to 1 per cent. of dextrose in sterile solution to milk in ordinary test tubes. After 3 to 5 days the casein, if not already precipitated through acid production, may be precipitated by immersing the tubes in hot water. The typhoid bacillus also acts upon the muscle sugar contained in beef which finds its way into bouillon.

May 30. A nine-day culture of typhoid III. in ordinary peptone bouillon with an initial acidity of 1.5 has a reaction for the open bulb of + 0.35; for the branch, — 2.64. Here we note the alkali production, which amounts to 2.64 + .35 or 2.99 per cent. The acid production is equal to 2.64 — 1.5 or 1.14 per cent.

The absence of acid production in the presence of saccharose and lactose in dextrose-free bouillon is shown by the following figures:

Saccharose bouillon, — 1.3[1] $\begin{cases} \text{bulb, — 0.28.} \\ \text{branch, — 1.30.} \end{cases}$

Lactose bouillon, — 1.3 $\begin{cases} \text{bulb, — 0.15.} \\ \text{branch, — 1.36.} \end{cases}$

Here the reaction of the branch fluid remains nearly unchanged, because the absence of an available sugar prevents growth without oxygen. In the bulb, however, there is active growth with alkali production.

We are now prepared to return to milk cultures. In order to bring out certain features of these more distinctly I have drawn into the study several varieties of hog-cholera (swine pest) bacilli, a bacillus of pseudo-tuberculosis described in this journal (vol. I., no. 16, p. 12) and closely related to the preceding, and a fowl disease bacillus[2] which resembles the typhoid bacillus closely, but which is non-motile. These were the only bacteria at my disposal which act on dextrose, but do not act on lactose. Those which do, like the large colon group, cannot be utilized. Nor can bacteria be used which do not act on dextrose, like many

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1 Initial reaction of bouillon.

2 V. A. Moore. Rep. Bur. Animal Industry for 1895–6, p. 188.

strictly aërobic forms. A few illustrations of milk cultures of these bacilli in fermentation tubes will make their action clear.

4-day culture of typhoid I., — 1.74 $\begin{cases}\text{bulb, — 3.18.}\\ \text{branch, — 3.06.}\end{cases}$

4-day culture of hog-cholera I., — 1.74 $\begin{cases}\text{bulb, — 0.64.}\\ \text{branch, — 3.19.}\end{cases}$

5-day culture of typhoid II., — 2.04 $\begin{cases}\text{bulb, — 3.07.}\\ \text{branch, — 3.04.}\end{cases}$

5-day culture of typhoid III., — 2.04 $\begin{cases}\text{bulb, — 2.95.}\\ \text{branch, — 2.92.}\end{cases}$

9-day culture of pseudotuberculosis, — 2.04 $\begin{cases}\text{bulb, — 0.3.}\\ \text{branch, — 3.08.}\end{cases}$

9-day culture of fowl disease, — 2.04 $\begin{cases}\text{bulb, — 0.43.}\\ \text{branch, — 3.12.}\end{cases}$

9-day culture of typhoid I., — 1.66 $\begin{cases}\text{bulb, — 3.07.}\\ \text{branch, — 2.91.}\end{cases}$

9-day culture of typhoid III., — 1.66 $\begin{cases}\text{bulb, — 2.81.}\\ \text{branch, — 3.03.}\end{cases}$

9-day culture of hog-cholera II., — 1.66 $\begin{cases}\text{bulb, — 0.}\\ \text{branch, — 2.68.}\end{cases}$

An examination of these figures will show that the amount of acid produced in the closed branch is nearly the same for all the bacteria under observation. In the bulb, however, the conditions are different. While the related bacilli produce considerable alkali, as shown by the fall in the acidity, the typhoid cultures produce no alkali. The reaction remains stationary after the acidity has reached its maximum.[1] If the acidity of these cultures were due to lactose, it is quite difficult to understand why so little acid is formed in the presence of so much sugar, and also why the same amount is formed by all cultures alike. I have already pointed out that if dextrose be added to milk, acid production goes on

[1] The slight decrease in the acidity of the last culture is probably due to slight interchanges of fluid between bulb and branch. The greater the difference of reaction between the two branches of the tube, the more conspicuous this defect becomes. When gases are formed the fluctuations of volume due to opening and closing of the incubator and to removal of the tube to a lower temperature for examination will reduce the acidity of the branch quite appreciably by drawing fluid from the open into the closed arm and forcing it out again.

to the precipitation of casein. The simplest explanation is the presence of very small amounts of dextrose. The uniform results quoted above apply to four different lots of milk from two dairies, and cannot therefore be looked upon as accidental. The fact that typhoid cultures remain acid is probably due to the unfavorable character of milk as a culture fluid, since alkali is produced abundantly in bouillon.

If a fermentescible carbohydrate, such as dextrose, is present in milk, it would be likely to appear in milk-sugar in traces. More than this is not to be anticipated, since the greater solubility of dextrose would lead to its elimination during the crystallization of the lactose. The following 7-day cultures of typhoid bacilli show that with an increase in the quantity of lactose there is a corresponding increase of acid:

Dextrose-free bouillon (control-tube), — 1.5 $\begin{cases} \text{bulb, } -0.86. \\ \text{branch, } -1.59. \end{cases}$

1.8 per cent. lactose bouillon, — 1.5 $\begin{cases} \text{bulb, } +0.05. \\ \text{branch, } -1.63. \end{cases}$

4.1 per cent. lactose bouillon, — 1.5 $\begin{cases} \text{bulb, } +0.05. \\ \text{branch, } -1.94. \end{cases}$

6.66 per cent. lactose bouillon, — 1.5 $\begin{cases} \text{bulb, } +0.0. \\ \text{branch, } -2.1. \end{cases}$

The following 5-day hog-cholera cultures furnish the same evidence. In the second and the third tube a gas bubble appeared, as a further indication of the presence of dextrose:

1.8 per cent. lactose bouillon, — 1.5 $\begin{cases} \text{bulb, } +0.15. \\ \text{branch, } -1.67. \end{cases}$

4.1 per cent. lactose bouillon, — 1.5 $\begin{cases} \text{bulb, } -0.10. \\ \text{branch, } -1.94. \end{cases}$

6.66 per cent. lactose bouillon, — 1.5 $\begin{cases} \text{bulb, } -0.38. \\ \text{branch, } -2.06. \end{cases}$

The presence of another carbohydrate in milk is merely hinted at in works I have been able to consult. Nothing definite seems to be known about it, and it is usually ignored.[1] That dextrose should be present is not strange,

[1] See Hammarsten Physiol. Chemie. (1895), p. 386.

but rather to be anticipated, as it is an acknowledged constituent of tissues and organs. In normal urine Baumann and Wedenski found about 0.09 per cent.[1] An acid-producing substance is readily demonstrated with the fermentation tube in animal tissues. By forcing bits of sterile tissue, as large as peas, from animals just chloroformed, into the closed branch of tubes containing dextrose-free bouillon, and inoculating with B. coli, or related forms, I obtained the following figures after 6 days:

Spleen of guinea-pig (B. coli), — 1.55 { bulb, + 0.3. branch, — 2.27.

Spleen of guinea-pig (hog-cholera bacilli), — 1.55 { bulb, + 0.1. branch, — 2.04.

In order to determine approximately the amount of the dextrose-like body present in milk, different quantities of dextrose[2] in sterile solution were added to dextrose-free bouillon in fermentation tubes and the series inoculated with typhoid III. After 3 days the following condition was observed:

Control tube, — 1.32 { bulb, — 0.44. branch, — 1.11.

Bouillon plus 0.05 per cent. dextrose, — 1.32 { — 0.3. — 1.89.

Bouillon plus 0.075 per cent. dextrose, — 1.32 { bulb, — branch, — 2.15.

Bouillon plus 0.1 per cent. dextrose, — 1.32 { bulb, — branch, — 2.47.

Bouillon plus 0.15 per cent. dextrose, — 1.32 { bulb, — 0.55. branch, — 3.03.

By comparing the increase in the acidity of the branch fluid in these tubes with that of the milk tubes tabulated above, we note that the amount of dextrose or related body in milk is about 0.1 per cent., or nearly the amount found in normal urine.

Petruschky found the amount of acid produced in litmus whey by typhoid bacilli 0.2 to 0.3 per cent. of a normal soln-

[1] Hoppe Seyler's Handbuch (1893), p. 60.

[2] The dextrose used in these experiments is the anhydrous powder.

tion. The amount produced in fermentation tubes is fully one per cent. The difference is probably due to the methods employed. Petruschky classed the hog-cholera bacillus as an alkali producer, the typhoid bacillus as an acid producer. The figures I have given both explain and destroy his classification. It would now be nearer the truth to state that both are acid producers, but that only one is an alkali producer in milk.

Another method, introduced by Prof. W. H. Welch, consists in the use of milk to which litmus has been added. With this modified milk, acid production by typhoid bacilli is evidenced by a change in color within 48 hours. The hog-cholera group of bacteria also shows this change, but it soon becomes confused with a reducing process which after 5 or 6 days leads to a complete but temporary decolorization of the litmus excepting at the surface. This reducing action is not noticed in milk cultures of typhoid or the fowl disease bacilli. In about 10 days the color returns to the hog-cholera tubes which have in the meantime become alkaline, while the typhoid cultures still show a reddish tint of the litmus. The reducing action seems to be due simply to a greater vigor of growth. The returning color (oxidation) indicates a slackening of the growth of the bacilli which enables the oxidizing action of the air to overshadow the enfeebled reducing action of the bacteria.[1]

There can be no question that the absence of alkali-production in milk is an important character of typhoid bacilli, and should not be neglected when questions of identity arise. Petruschky has emphasized this point in the description of a bacillus from feces which resembles the true typhoid bacillus very closely, but produces alkali.[2] Though the serum test has now entered as the most weighty evidence in deciding whether suspected bacilli are typhoid or not, it would be highly unwise and misleading to rely upon this alone and

[1] Theobald Smith. Reduktionserscheinungen bei Bakterien. Centralblatt f. Bakt., XIX. (1896), p. 181.

[2] *Bacillus fæcalis alcaligenes*. Centralblatt f. Bakt., XIX., p. 187. See also Capaldi and Proskauer. Beiträge z. Kenntniss d. Säurebildung bei Typhusbacillen u. Bact. Coli. Ztschr. f. Hygiene, XXIII. (1896), p. 452.

neglect to study the important characters of the species. All avenues for the detection of variations would thereby be blocked, while, at the same time, the significance of the agglutinative action of the blood as manifested towards different species of bacteria would continue to remain a matter for speculation.

For acid and alkali production I would recommend the use of milk in fermentation tubes and the titration of the fluid in .open and closed arms. This process is superior to the use of litmus whey and litmus milk, since it gives quantitative results and employs a more reliable indicator. Litmus milk is serviceable in supplementing this method, as it shows to the eye changes of reaction and indicates by the phenomena of reduction the relative vigor of multiplication and the time when this begins to languish and cease. Uniform narrow tubes should be used, and the column of liquid should be of the same height if the phenomena of reduction are to have any comparative value. The main points of this article may be epitomized as follows:

1. Typhoid bacilli produce alkali in bouillon, but not in milk.

2. Typhoid and closely related bacilli which do not coagulate milk (do not act on lactose) produce an equal amount of acid in this fluid.

3. In milk there is a substance, present to about 0.1 per cent., which resembles dextrose in its behavior towards bacteria.

4. The comparative slowness with which changes of reaction take place in milk cultures indicates that it is a less favorable medium for certain bacteria than peptone bouillon.

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